

Rapid Acetylation of Alcohols at the Microgram Level Using a Celite-Acetyl Methanesulfonate Column

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Received April 11, 1975; accepted October 15, 1975

A simple procedure is described in which micro- and submicrogram amounts of primary, secondary, and several tertiary alcohols can be acetylated. The alcohols, dissolved in a nonpolar solvent, contact a column of Celite charged with the mixed anhydride, acetyl methanesulfonate, contained in a melting-point capillary. The resulting acetate is then eluted for analysis. The entire procedure can be executed in less than a minute. Micromole amounts of alcohols can be acetylated in good yield using larger columns.

Previous papers from this laboratory have described simple column procedures for conducting oxidations (1–3), reduction (4), methylation (5), hydrogenation (6), and adduct formation (7) on microgram and/or micromole amounts of appropriate substrates. The present report is concerned with an acetylation technique performed rapidly on a column of Celite charged with the mixed anhydride, acetyl methanesulfonate. Mixed sulfonic acid–carboxylic acid anhydrides have been shown to be potent acylating reagents in homogeneous solution on the macro- and semimacro scale (8–12). A column of cross-linked polystyrene resin containing acetyl benzenesulfonate units has been used to acetylate some simple alcohols at the semimacro scale (13).

MATERIALS

Preparation of impregnated Celite A. Celite 545 (Johns Manville Co.,² Baltimore, Md.) was dried at 500–600°C for 18 hr. The dried Celite (4 g) and 1 g P₂O₅ (added to try to maintain anhydrous conditions) were ground together in a mortar, then stored overnight in a desiccator containing P₂O₅. The Celite–P₂O₅ powder (5 g) and 1 ml acetyl methanesulfonate (Willow Brook Laboratories, Waukesha, Wis.) were ground in a 4 in. mortar until homogeneously tan. It is preferable to do this in a dry box or, if none is available, rapidly on a low-humidity day. The acetylating powder

¹ Agricultural Research Service, U.S. Department of Agriculture.

² Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

(designated Celite A) is stored at -18°C over P_2O_5 when not in use. Under these conditions, it remains active for at least 1 yr.

Two variations for preparing the acetylating powder were investigated. These were Celite B, prepared the same way as Celite A, but the P_2O_5 was omitted; and Celite C, prepared the same way as Celite A, except that the Celite was dried at 140°C instead of at $500\text{--}600^{\circ}\text{C}$. Both powders appeared to be just as efficient as Celite A. However, they were prepared toward the end of the study and not evaluated beyond 2 mo.

EXPERIMENTAL

Microacetylation procedure. The end of a melting point capillary (about 50 mm long \times 1.5–2.0 mm o.d.) was dabbed into the acetylating powder until a column approximately 1.5 cm in length was retained. The powder was pushed into the capillary with the straight end of a paper clip or other suitable tamper and held there while the powder was compressed into a compact column about 1 cm in length with another tamper. From 0.2 to 200 μg^3 of the alcohol (usually 1–3 μg in this study) dissolved in 1 to 10 μl of CCl_4 or other suitable solvent (see Discussion) was transferred to the column with a hypodermic syringe. The sides of the capillary were washed down with 2 μl of solvent and the column was eluted with a column volume of solvent using light air or nitrogen pressure if desired.⁴ The effluent was removed as thoroughly as possible with a hypodermic syringe. If pressure was used, the entire operation could be done in less than 1 min.

Acetic acid and acetic anhydride emerged in the effluent along with the acetate. If these interfered in subsequent analysis of the effluent, they were removed easily as follows: After the sample had been applied to the acetylation column, the exit end of the capillary was dabbed into Celite impregnated with dibasic potassium phosphate⁵ until about 1.5 cm of the powder was retained. The powder was pushed against the acetylation column and the columns were eluted with a volume of solvent approximately equal to the length of the two columns.

Analysis of effluent. The effluent from the column was analyzed by injecting it directly into a gas chromatograph–mass spectrometer. The following conditions prevailed: Instrument, LKB-9000 operating at an ionizing energy of 70 eV, accelerating voltage 3.5 kV, separator temperature, 240°C , and ion source temperature, 290°C . Chromatographic columns were: (a) 5 ft \times $\frac{1}{8}$ in. (o.d.) silanized stainless steel packed with 7.5%

³ These figures were established for 4-methyl-4-nonanol. The upper limit was not ascertained.

⁴ If air pressure is to be used, a convenient apparatus is a rubber bulb with a netted reservoir and connecting tube which contains at its end a rubber capillary holder such as that supplied with Microcaps.

⁵ A saturated solution of K_2HPO_4 (1 ml), about 1 mg of Phenol red, and 2 g of Celite 545 are ground together in a mortar until homogeneously purple.

ethylene glycol adipate and 2% phosphoric acid on 90–100 mesh Anakrom ABS programmed from 70 to 190°C at 2 or 4°C/min; (b) 4 ft × 1/8 in. silanized stainless steel packed with 3% OV-225 on 100–120 mesh Gas-Chrom Q programmed from 70 to 220°C at 2 or 4°C/min; and (c) 4 ft × 1/8 in. (o.d.) silanized stainless steel packed with 1.5% OV-101 on 100–120 mesh Gas-Chrom G-H.P. programmed from 70 to 250°C at 2 or 4°C/min. Helium was the carrier gas in all cases and was supplied at a head pressure of 40 psi.

In a number of instances, retention times of authentic acetates prepared by classical procedures were compared with those made on the acetylating column.

Acetylation at the micromole level. Although the prime objective of the work was to develop a rapid, simple acetylation technique for micro- and submicrogram amounts of alcohols, a limited study of running micromole amounts of alcohols over larger columns was also carried out. At the same time the use of larger amounts was more amenable to quantitative analysis. The powder (200 mg ± 5 mg of Celite A or B) was transferred to a Pasteur pipet (145 × 7 mm o.d.) cut just below the crimp and plugged with glass wool. The powder was tamped lightly so that 1 ml of CCl₄ flowed completely into the column in about 3 min. One milliliter of a CCl₄ solution of the alcohol was pipetted onto the column, and the effluent was collected immediately in a 2-ml volumetric flask. The column was washed with CCl₄ until 2 ml were collected. Aliquots of the effluent were analyzed by quantitative glc using pure acetates to establish standard curves.

RESULTS AND DISCUSSION

The alcohols passed over the microcolumns are listed in Table 1 together with the product that was identified in the effluent. With the exception of cinnamyl, α -methylcinnamyl, furfuryl, *o*- and *p*-methoxybenzyl, and triphenylmethyl alcohols, all compounds including the tertiary alcohols gave the corresponding acetates. No trace of starting alcohol was observed even with the alcohols that did not give an acetate. All of the alcohols listed above with the exception of the highly hindered triphenylmethanol gave acetates with acetic anhydride and pyridine (1:1). They also gave, with the exception of the acid-sensitive furfuryl alcohol and triphenylmethanol, an acetate using a perchloric acid catalyzed procedure (14). The reason for the failure of the cinnamyl alcohols and the *o*- and *p*-methoxybenzyl alcohols to form an acetate on the column is not clear, but it is possible that selective ring acylation occurred (15).

Solvents. Besides CCl₄, a number of other solvents were checked in a limited number of trials. Successful acetylation was carried out with CS₂, hexane, petroleum ether, benzene:CCl₄ (9:1), and with CCl₄:CH₂Cl₂ (8:2). Solvents more polar than the latter start to elute color from the

TABLE 1
ALCOHOLS EXAMINED ON AN ACETYLATED COLUMN OF ACETYL
METHANESULFONATE ON CELITE 545

Alcohol	Corresponding acetate formed	Gas chromatographic column used ^a
Behenyl alcohol	+	b
Benzyl alcohol	+	a
2-Butoxyethanol	+	a,c
3 β -Cholesterol	+	b
3 β -Cholesterol	+	b
Cinnamyl alcohol	—	a
1,2-Cyclohexanediol	+	b
3-Cyclohexen-1-methanol	+	a
Cyclohexylmethanol	+	a
2,2-Dimethyl-3-octanol	+	a
11,14-Eicosadien-1-ol	+	c
3-Ethyl-3-pentanol	+	a,c
Furfuryl alcohol	—	a,c
2-Hexyl-1-decanol	+	a
18-Hydroxypentatriacontane	+	b
Lanosterol	+	b
1-Menthol	+	a
<i>o</i> -Methoxyphenol	+	b
<i>m</i> -Methoxyphenol	+	b
<i>p</i> -Methoxyphenol	+	b
<i>o</i> -Methoxybenzyl alcohol	—	a,b,c
<i>m</i> -Methoxybenzyl alcohol	+	a,b,c
<i>p</i> -Methoxybenzyl alcohol	—	a,b,c
α -Methylcinnamyl alcohol	—	a
Methyl-9(10)-hydroxymyristate	+	b
Methyl-6-hydroxystearate	+	b
2-Methyl-2-nonanol	+	a,b
2-Methyl-4-nonanol	+	a,b
4-Methyl-4-nonanol	+	a,b
1-Naphthol	+	a
1-Octadecanol	+	b
Phenol	+	a
1-Phenyl-1-propanol	+	a
Triphenylmethanol	—	b,c

^a (a) EGA-H₃PO₄; (b) OV-225; (c) OV-101.

Celite. The limitation posed by the polarity of the solvent precludes the use of the column to acetylate the majority of polyhydroxy compounds.

Quantitative aspects. No effort was made to try to quantitate the acetate in the effluent of the microgram level study. Judging roughly from the peak heights of the acetates of primary and secondary alcohols,

it appeared that yields were good. It was noted, however, in some instances with the tertiary alcohols, that yields might not be as good as they were with the primary and secondary alcohols.

For the trials conducted at the micromole levels, the yields of acetates were nearly quantitative. The following alcohols were assayed, and the amount that went over the column and the yield of acetate as determined by quantitative glc is given: 1-octadecanol, 11.7 μmol (100%); 1-tetradecanol, 10.1 μmol (97%); phenol 13.3 μmol (102%); 1-naphthol, 8.2 μmol (95%).

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